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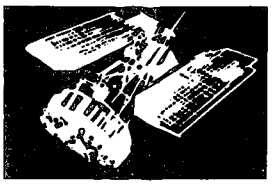
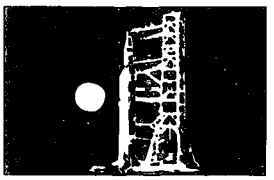
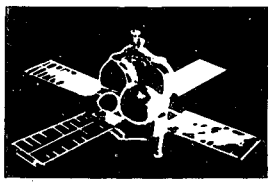

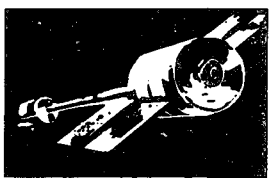
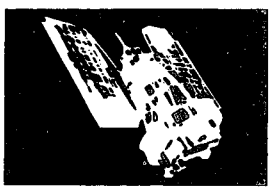
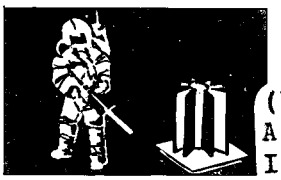
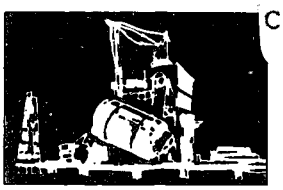

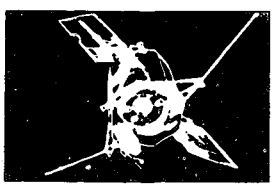

IMBLMS
PHASE B4

ADDITIONAL TASKS
TASK 5.0

Microbial Identification System
FINAL REPORT

IMBLMS

ORIGINAL CONTAINS
COLOR ILLUSTRATIONS

Contract NAS 9-10741
Phase B4

GENERAL ELECTRIC

G.E. No. 70SD5414
October 29, 1971

FINAL REPORT

FOR

TASK 5.0

MICROBIAL IDENTIFICATION SYSTEM

IMBLMS PHASE B-4

ADDITIONAL TASKS

CONTRACT NAS9-10761

GENERAL ELECTRIC COMPANY

SPACE DIVISION

MICROBIAL IDENTIFICATION SYSTEM

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1.0 SUMMARY

A laboratory study was undertaken to provide simplified procedures leading to the presumptive identification (I/D) of defined micro-organisms on-board an orbiting spacecraft. Identifications were to be initiated by non-professional bacteriologists, i.e., crew members, on a contingency basis only. Key objectives/constraints for this investigation were as follows:

- o I/D procedures based on limited, defined diagnostic tests
- o Testing oriented about ten selected micro-organisms
- o Provide for definitive I/D key and procedures per selected organism
- o Define possible occurrences of false positives for the resulting I/D key by search of the appropriate literature.
- o Evaluation of the I/D key and procedures through a limited field trial on randomly selected subjects using the I/D key.

All objectives were met during the course of the investigation. In addition, a series of color photographs were taken of all significant reactions to each diagnostic test performed on the array of selected micro-organisms to provide supportive laboratory records and (potentially) serve as an aid during in-flight use.

Results of the investigation indicate that the defined tests are adequate for implementation of the I/D Key except for 4 of the 10 selected microorganisms (the Enterbacteriaciae) which cannot be identified unambiguously without the addition of one step for further biochemical tests, e.g., the Roche "Enterotube". The possibility of the occurrence of false positives was considered to be minimal with the significant exception of the same group cited above. Results of the field test using the I/D Key Nod Procedures was judged a qualified success due primarily to the limited number of subjects tested (13), and the decision (reflected in the Statement of Work) to omit fecal samples from the testing protocol.

Recommendations include:

- The insertion of the Roche "Enterotube" into the array of diagnostic tools.
- Emphasis on microscopic training as a critical skill in implementing the I/D key.
- A study of the critical amount of cellular material required to eliminate false negative responses to the catalase test.
- More extensive, additional testing of live subjects using I/D key procedures.

2.0 PROLOGUE

2.1 Problem Definition

Future manned spacecraft missions which call for extended flight times, i.e., more than two weeks, must be prepared for the necessity of dealing with possible microbiological problems. At the very least, scientists and medical observers on the ground will want a timely and representative sample from infections, rashes, etc., of suspected microbial origin. Optimally, the means to perform a presumptive identification of the possible causative agent should be available for on-board use. This also implies that spacecraft crew members, who are not likely to be trained microbiologists, will be called upon to perform these isolations/identifications. The approach to these problems suggests two basic questions, which in turn forms the foundation of this investigation.

1. What is the largest number of medically significant micro-organisms which can be presumptively identified with a minimum number of tests/procedures?

2. How can non-professional bacteriologists, i.e., crew members, be utilized to obtain meaningful samples and relevant information regarding incipient microbiological problems with a minimum amount of pre-flight training and in-flight time?

A presumptive answer to the first question was supplied by the Work Statement which defined ten micro-organisms and a set of diagnostic tests and/or tools. These organisms and diagnostic tests are listed in the following sections.

2.1.1 Selected Micro-organisms

a. Gram Positive Cocci (spheres)

   1. Streptococcus pyogenes

   2. Staphylococcus aureus

   3. Diplococcus pneumoniae

b. Gram Negative Rods

Enterobacteriaciae

4. Salmonella typhi

5. Shigella dysenteriae

6. Proteus morganii

7. Enterbacter aerogenes

- -

8. Pseudomonas aeruginosa

9. Haemophilus influenziae

Other

10. Candida albicans - fungus which stains strongly Gram positive

2.1.2 Diagnostic Tests/Tools

1. Blood Agar Plates - primary isolation of initial samples and visual observation of colonial morphology

2. Taxo Discs

   - o Type "A" for identification of beta-hemolytic strep
   - o Type "P" for differentiation of the penumococci

3. Calalase Test - useful for biochemical separation of genera, especially strep and staph

4. Cytochrome - oxidase Test - used principally to separate Pseudomonal spp. from the Enterobacteriaciae

5. Gram stain - classical staining technique which, in conjunction with a microscope, divides the bacterial supp. into 4 broad categories on the basis of color uptake and cellular morphology (rod or sphere)

6. Antibiotic Sensi-discs

   - o Ampicillin - 10 mcg
   - o Gantrisin - 2 mg
   - o Furadantin/Macrodantin - 300 mcg
   - o Neomycin - 30 mcg
   - o Penicillin - 10 units
   - o Tetracycline - 30 mcg

2.2 Approach to the Problem

The attempt to answer the second question (2.1) forms the basic concept behind the approach to the problem and establishes the framework for the laboratory investigation described within this report. The exercise of each diagnostic test/procedure, in conjunction with the 10 selected organisms, is standard laboratory routine; the overall results of each test are obviously known in advance. Actual repetitive execution of these tests in the laboratory provides a better "feel", not only for the most efficient order of performance, but also for the objective criteria/ reactions most likely to be meaningful or obvious to non-professional bacteriologists. The key to the success of the entire operation depends, ultimately, upon the extrapolation of the objective observations received from the spacecraft by professional bacteriologists (in Mission Control) who are equipped with the same I/D Key and Procedures as the crew member/operator. The end product of this trade-off can result in a reasonable assessment of how to meet each situation.

Therefore, the laboratory study was begun with the following major objectives:

2.2.1 Binomial Pathway for Presumptive I/D

Presumptive identification of each of the 10 selected organisms was to be organized along a "bionomial pathway". At each decision point, a "yes" or "no" answer would lead logically to the next step until all 10 organisms were separated and/or identified. This goal is interdependent with the next two objectives.

2.2.2 Literature Search for Possible False Negatives

It was recognized that bacteria/fungus of medical significance, other than the 10 listed above, could be separated by the same diagnostic test procedures. What are these organisms, and how can their interference be identified or at least recognized? A search of method's text books, i.e. medical diagnosis, procedures manuals, etc. would be needed to uncover those organisms which can be associated with the occurrence of false positives.

2.2.3 Simplified "Cookbook" Procedures For Operator

Implementation of the presumptive I/D Key would be through a simple check list procedure. This will serve two purposes: (1) provide a training base for future operators, and (2) provide a basis for comparison by Mission Control experts to aid in this interpretation.

2.2.4 Color Photographs of Each Selected Organism versus Significant Diagnostic Tools

It was an objective to obtain color photographs of significant reactions to diagnostic tests performed on the selected micro-organisms, both to provide a permanent record supporting the findings, and potentially to serve as a common objective and for comparison between the on-board operator and Mission Control experts.

2.2.5 Evaluation of I/D Key by Live Testing of Proposed I/D Procedures

Once the I/D Key has been established and the corresponding procedures for its implementation have been written, an evaluation of the entire process will be undertaken. Due to budgetary limitations, it was decided at the outset that fecal samples would not be included in the testing procedure and that the number of random subjects tested would be limited to ten. Three samples: throat, nares, and skin, would be obtained from each subject and then subjected to the proposed I/D procedures for a total of 30 samples. Two obvious limitations were recognized:

- Ten subjects represent a statistically marginal number with which to attempt an evaluation of the I/D key, and
- Omission of fecal samples from each of the subjects precluded the possibility of the presumptive identification of Enterobactericaciae (4 organisms) during testing.

However, it was felt that sufficient information could be gleaned from the exercise to obtain an objective assessment of procedural feasibility and false positives along with the limited presumptive identifications.

3.0 PREPARATORY LABORATORY EFFORT

Before the defined diagnostic tests could be applied to each of the 10 selected organisms, it was necessary to establish those cultures in an actively metabolizing state, or "biochemical maturity", in order that reproduceable, predictable reactions may be expected to each test procedure.

Stock Cultures - Growth and Assay

The following organisms, designed by the Work Statement, were ordered from the American Type Culture Collection (ATCC)[1]

	Micro-organism	ATCC #
1.	Proteus morganii	9237
2.	Enterobacter aerogenes	13048
3.	Pseudomonas aeruginosa	17423
4.	Streptococcus pygoenes	14289
5.	Staphylococcus aureus	6339
6.	Diplococcus pneumoniae	9163
7.	Haemophilus influenziae	8149
8.	Candida albicans	10231
9.	Salmonella typhi	6539
10.	Shigella dysenteriae	13313

Procedures used in the rehydration and quantitative assay of the marker organisms are detailed in the Appendix, Sections 6.1 and 6.2. All cultures, except H. influenziae, were subcultured 6-8 times in either nutrient broth or Trypticase-Soy broth. Plate count assays were performed on the last 2 or 3 subcultures or until the titers appeared stable. H. influenziae demonstrated some reluctance to grow in submerged culture, and was carried by loop transfer on Blood Agar plates (Typicase-Soy agar plus 5% sheep blood). H. influenziae subsequently produced growth in Brain Heart Infusion Broth plus Bacto-Supplement B (sterile yeast concentrate), but

[1] American Type Culture Collection 12301 Parklawn Drive, Rockville, Md. 20852

quantitative plate counts were not carried out. Table 3.1-1 summarizes the results of quantitative assays on 18 hour cultures of the final liquid passage.

As inferred from Table 3.1-1, the titer of each test organism, except H. influenziae, was considered satisfactory for initiation of the diagnostic testing. The cultures were considered "biochemically mature" i.e., likely to react in similar fashion as the naturally occuring organisms after isolation from human infections.

TABLE 3.1-1 RESULTS OF QUANTITATIVE PLATE COUNTS ON SELECTED TEST ORGANISMS* FOLLOWING 18-Hr. GROWTH IN LIQUID MEDIA AT 37°C

Organism	Growth Medium	Passage Number	Titer
Strep. pyogenes	Tryp. Soy Broth	8	1.9×10^8
D. pneumoniae	" " "	6	4.5×10^7
C. albicans	Nutrient Broth	7	1.4×10^6
P. morganii	" "	6	1.7×10^8
Entero. aerogenes	" "	6	4.0×10^8
P. aeruginosa	" "	6	6.8×10^8
Staph. aureus	" "	6	3.5×10^7
Sal. typhi	" "	6	4.0×10^7
Sh. dysenteriae	" "	6	1.8×10^7

* H. influenziae not assayed

4.0 RESULTS OF THE INVESTIGATIONS

4.1 Identification (I/D) Key

The routine diagnostic tools listed in Section 2.1 were tested in the laboratory against each organism. Details of the diagnostic test procedures are given in the Appendix, Section 6.3. The observed reactions were evaluated in terms of the most relevant and objective criteria, and in terms of the optimum order in which to perform each test. The end result of these evaluations is the I/D Key, shown in Figure 4.1-1.

As discussed in Section 2.2, the presumptive I/D Key follows a binominal pathway. This allows the operator to perform a series of objective tests, i.e., positive or negative; yes or no, which successively eliminates possibilities until a single organism, or group of organisms, can be presumptively identified. As implied in Figure 4.1-1, separation of most of the 10 selected micro-organisms from each other is straight-forward, with the exception of 3 genera of bacteria belonging to the family Enterobacteriaciae. Within the limitations imposed by the diagnostic tools defined for this study, no macro or microscopic examination or biochemical test is available for separating and identifying these genera. The separation/identification problem becomes even more actue when Shigella spp. other than S. dysenteriae are encountered; all other Shigella spp. are catalase positive. This holds true for the 5 major divisions, involving 11 genera of bacteria, which encompass the Enterbacteriaciae. This situation, together with the general consideration of false positives (with respect to the 10 selected organisms) is discussed in more detail in Section 4.3.

The Gram stain is an extremely important cog in the chain of tests used to separate these organisms and/or detect others with similar reactions (false positives). Standardized, i.e., consistant, Gram stain technique, therefore, is very important. Examination of Gram stained slides must be

done carefully and cuatiously in order to avoid possible confusion or misinterpretation from artifacts. This is especially true with stains from possible fungus isolates where staining characteristics tend to vary as the age of the sample and which display a high incidence of pleomorphism as well. For these reasons, emphasis on microscopic training is strongly recommended.

The nasopharyngial "Calgiswab" (thin)[2] was used to transfer single colonies of each organism to the Blood Agar (BA) isolation plate. Thus, all tests were originated from a single, isolated colony. The "Caligswab" proved to be almost as easy to use and manipulate as the standard bacteriologist's loop. Some difficulty was experienced when colony separation was poor; i.e., adherence of cellular material from other colonies to the swab was difficult to prevent when colonies were too close together.

Satisfactory performance of the catalase test is also closely associated with the "Calgiswab". Positive test results depend upon the observation of small bubbles when 3% hydrogen peroxide is added to cellular material from the sample organism. Judgement as to the presence or absence of these bubbles depends directly on the amount of cellular material available, either on a strip of filter paper, or directly on the sample swab placed in a test tube containing H_2O_2. Experience in this laboratory has indirected that critical amounts of test sample, i.e., cellular material from isolated colonies, is necessary to avoid possible false negative judgements when performing the test. This problem impinges directly upon the degree of colony separation demonstrated by the growth on the BA isolation plate. Therefore, it is recommended that some attention be directed toward defining, as objectively as possible, the amount of sample material required to produce an unambiguous result during performance of the catalse test.

[2] Ordered from Colab Laboratories Inc., 3 Science Road, Glenwood, Ill. 60425. Stock Nos. 11-60-5 (thin); 11-20-1 (bulky).

IDENTIFICATION KEY - FLOW DIAGRAM

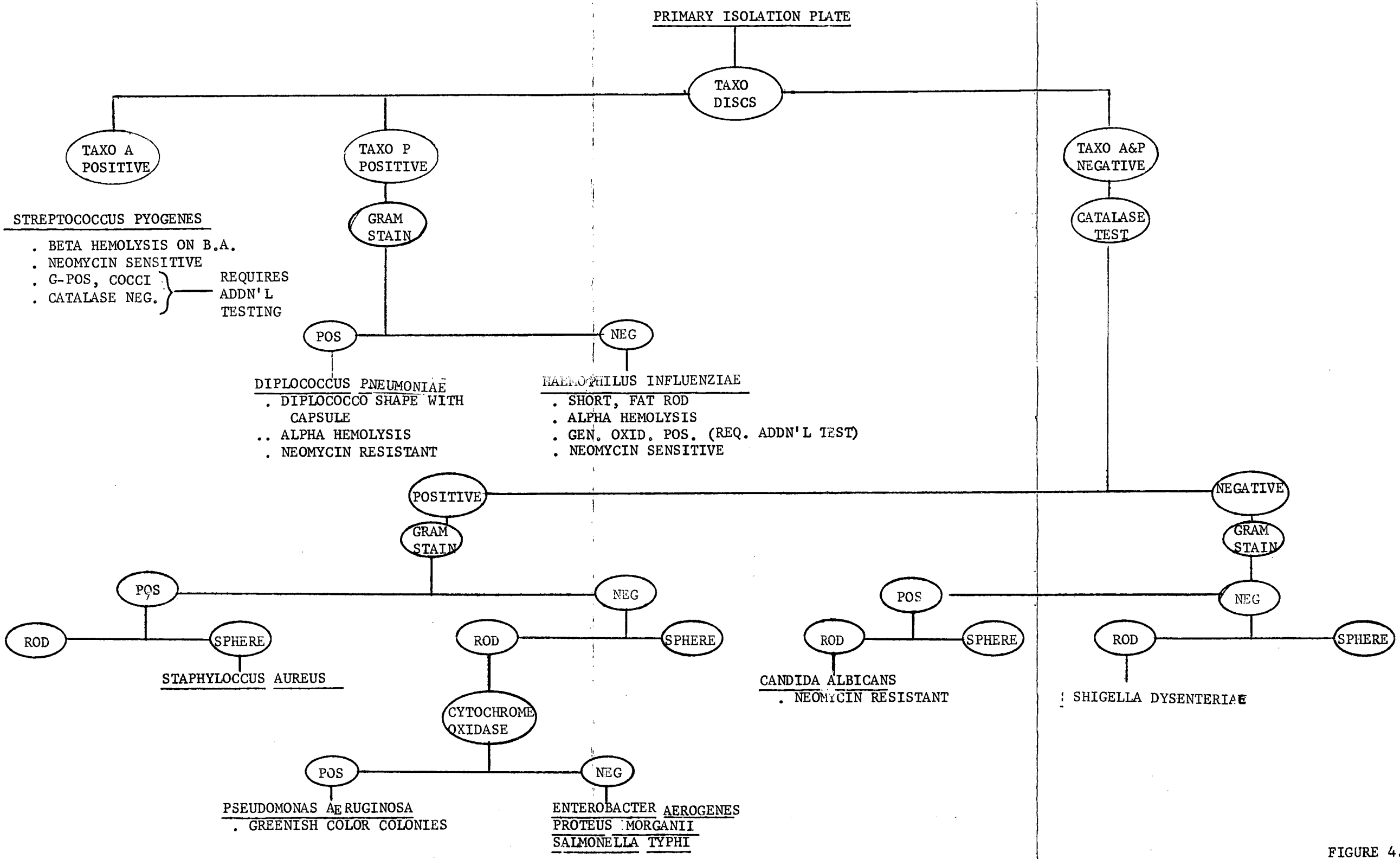


FIGURE 4.1-1

FOLDOUT FRAME 1

FOLDOUT FRAME 2

On BA plates, the demonstration of hemolysis, either alpha or beta, is an observable phenomenon useful in the separation of some stains of bacteria. It should be reported that contrary to published literature, H. influenzae consistently demonstrated alpha hemolysis as well as sensitivity to Taxo P discs during the laboratory investigation phase. Technical personnel from ATCC, from whom the stain was obtained, could offer no explanation of this apparent anomaly. Diplococcus pneumoniae, another bacterial strain within the 10 selected organisms which normally exhibits alpha hemolysis and sensitivity to Taxo P can be separated by Gram staining: Gram negative rods and Gram positive cocci respectively. The Neomycin sensi-disc (30 mcg) is also quite useful in distinguishing H. influenzae from D. pneumoniae; H. influenzae is Neomycin sensitive.

4.2 Simplified Procedure for Use With I/D Key

In order to implement the I/D Key, a step-by-step procedure was prepared to aid the operator in performing the presumptive identification. The basic outline, or flow chart, upon which these procedures were constructed, is displayed in Figure 4.2-1. As shown, Taxo A and P discs and a Neomycin disc are depositied on the BA isolation plate as the first step. With the appearance of isolated colinies (following incubation), a number of options are available:

- Determination of sensitivity to Taxo discs and/or Neomycin
- Macroscopic examination of colonial appearance on the Blood Agar plate - see Table 4.2-1.
- Performance of catalase and/or cytochrome-oxidase tests
- Gram staining of suitable preparation followed by microscopic examination.

The decisions as to when to perform these tests is controlled by the bionomial system inherent within the I/D Key.

Macroscopic examination and description of colonia morphology is recommended as an integral part of this procedure, both as a valuable adjunct to the objective data normally collected, and as common reference point for additional amplifying questions from Mission Control scientists. Table 4.2-1 is suggested as a possible model for describing those characteristics which might be discussed during visual examination of the primary isolation plate.

An additional recommendation concerns the use of a visual aid in accomplishing the macroscopic examination. Employment of a low power magnification system, e.g., 4 or 5 x magnifying glass, or optimally, a laboratory steroscope in conjunction with a maneuverable, variable intensity light source will substantially aid in providing higher quality information. Details which could be of critical importance frequently arenot apparent without optical assistance.

Subculture of single isolated colonies for determination of antibiotic resistance is made with the "Caligswab" (thin). By proper manipulation of the swab a contiguous lawn can be produced on a second BA plate. Placement of the antibiotic sensi-discs immediately following the transfer completes the task. Incubation for 18-24 hours will allow a roughly quantitative estimate of the degree of antibiotic resistance/sensitivity. Table 4.2-2 summarizes the results of 6 antibiotics at the stated concentration levels versus the 10 selected micro-organisms.

Figures 4.2-2, 3,4 outline the suggested complete procedure to be followed in conjunction with the I/D Key (Figure 4.1-1). It should be emphasized that Figures 4.2-2, 3, 4 represent a procedure rather than instructions per se.

TABLE 4.2-1

SUMMARY TABLE - COLONY CHARACTERISTICS/DIAGNOSTIC TEST REACTIONS

ON TEN SELECTED BACTERIA

CHARACTERISTIC	DESCRIPTION	STREP. PY.	DIPL. PNEU.	CAN. ALBICANS	PRO. MORG.	ENT. AEROG.	PS. AERUG.	STAPH. AUREUS	SAL. TYPHI.	SHI. DYS.	HAEM. INFL.	REMARKS
FORM	PUNCTIFORM	X	X								X	
	CIRCULAR		X	X	X	X		X	X<1 mm	X<1mm	X	
	FILAMENTOUS											
	IRREGULAR			X			X <1mm					
	SPINDLE											
	RHIZOID			BLEND TOGETHER								
MARGIN	ENTIRE	X	X	X		X		X	X	X	X	
	UNDULATE				X		X					
	LOBATE											
	EROSE											
	FILAMENTOUS											
	CURLED											
ELEVATION	FLAT					X						GENERAL-MAGNIFICATION AT THE DISECTING SCOPE LEVEL IS PROBABLY NEEDED FOR RELIABLE ASSESSMENT OF THIS CHARACTERISTIC (15-20 X)
	RAISED					X						
	CONVEX	X		X		X		X	X	X	X	
	UMBONATE		X		X		X					
OPTICAL CHARACTERS	OPAQUE					X WHITISH	X CENTER	X OFF-WH	X WH-GR	X CENTER		
	TRANSLUCENT						X EDGES		X EDGES	X EDGES		
	TRANSPARENT	X	X	X	X						X	
	OPALESCENT											
	IRIDESCENT											
OTHER	ODOR	NONE	NONE	NONE	STRONG STENCH	YES	GRAPE ODOR	NONE	NONE	NONE	NONE	
	HEMOLYSIS	BETA	ALPHA	----	-----	---	----	LIMITED β HEM. IMMED. VIC. OF COL.	---	----	ALPHA?	
	COLOR	COLORLESS	GREYISH	GREYISH	GR.-WH.	WHITISH	GREENISH	WHITE	GREYISH	WHITISH	COLORLESS	
GRAM REACTION		G-POS COCCI CHAINS	G-POS. DIPLOCOC. CAPSULE	G-POS PLEOMOR.	G-NEG. ROD	G-NEG. ROD	G-NEG. ROD	G-POS COCCI CLUMPS	G-NEG. ROD		G-NEG ROD SHORT, FAT	
CATALASE		NEG.	NEG.	N/T	POS.	POS.	POS.	POS.	POS.	NEG.	N/T	N/T = NOT TESTABLE
OXIDASE		NEG.	NEG.	N/T	NEG.	NEG.	POS.	NEG.	NEG.	NEG.	POS. GENERALLY	
TAXO DISC A		POS.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	
P		NEG.	POS.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.	NEG.		
SENSI-DISC N-30 (NEOMYCIN -30 MEG)		SENS.	INSENS.	INSENS.	SENS.	SENS.	SENS.	SENS.	SENS.	SENS. $\pm$	SENS.	

FOLDOUT FRAME 1

FOLDOUT FRAME 2

TABLE 4.2-2.

SUMMARY OF RESULTS OF REACTIONS OF SENSI-DISC TESTING OF SELECTED ORGANISMS

ORGANISM	AGENT/LEVEL					
	Grantrisin 2.0 mg G	Ampicillin 10 mg AM	Penicillin 10 units P	Tetracycline 30 meg Te	Macrodantin 300 meg F/M	Neomycin 30 mg N
Streptococcus pyogenes	Resistant	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Diplococcus pneumoniae	Sensitive	Sensitive	Sensitive	Sensitive	Intermediate	Intermediate
Candida albicans	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
Proteus morganii	Sensitive	Resistant	Resistant	Sensitive	Intermediate	Sensitive
Enterobacter aerogenes	Intermediate*	Intermediate	Resistant	Inermediate*	Sensitive	Sensitive
Pseudomonas aeruginosa	Intermediate*	Resistant	Resistant	Resistant	Resistant	Intermediate
Staphylococcus aureus	Intermediate*	Sensitive**	Sensitive**	Intermediate	Resistant	Sensitive***
Salmonella typhi	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
Shigella dysenteriae	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Sensitive
Haemophilus influenzae	Intermediate*	Resistant	Sensitive	Sensitive	Sensitive	Resistant

* Some Resistant Colonies
** Green Halo Around Zone
*** Halo at Edges

BASIC PROTOCOL FOR PRESUMPTIVE IDENTIFICATION OF ORGANISMS OF INTEREST

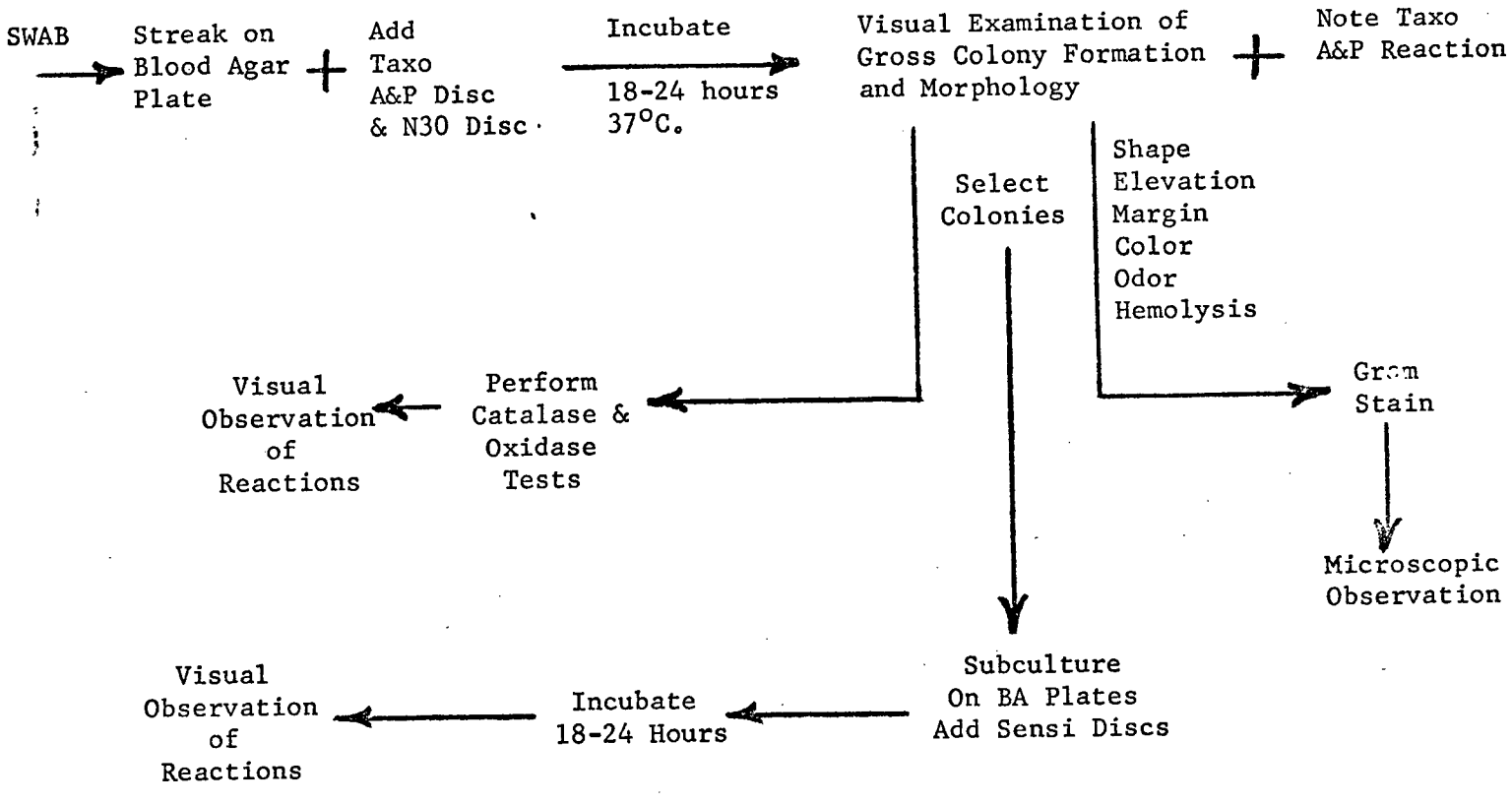
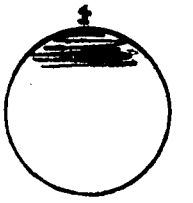


FIGURE 4.2-1

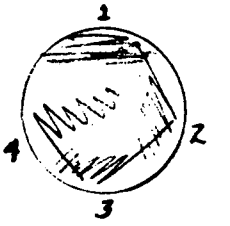
SIMPLIFIED PROCEDURES FOR USE OF I/D KEY

Figure 4.2-2 Phase I

1. Obtain swab sample.
2. Streak on upper portion of blood agar plate as shown:



3. Using 3 additional sterile swabs, make additional streaks as suggested by diagram:



4. Place Taxo A and P discs on opposite sides of a neomycin (30 MCG) sensi disc about 1 disc-diamter apart at the site of original streak:

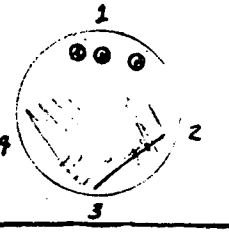


Figure 4.2-3 Phase II

1. Visually examine all incubated plates:
   - o using indicent and transmitted light without magnification
   - o using incident light with magnification levels of 10-20X

2. Estimate total number of different colony types. Record characteristics of each type noted according to diagram on pictorial I/D key.

3. Note and record reaction of each colony type to the Taxo discs:
   - o sensitivity of either Taxo disc to any colony type - go to I/D key
   - o for colony types demonstrating no sensitivity - go to next step

4. Perform Catalase Test on selected isolated colonies - record results

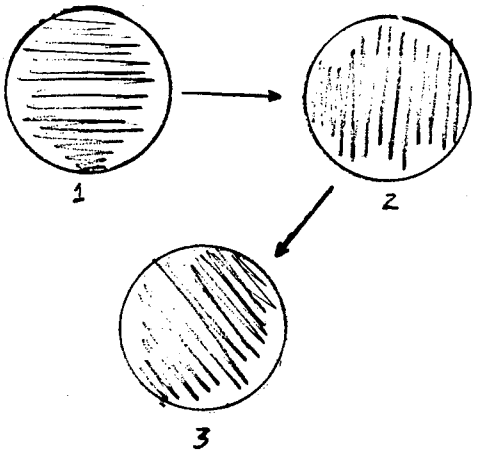
5. Prepare and examine microscopically Gram Stain smear of each colony type - record findings.

6. Consult pictorial and flow I/D key for correlation of findings and/or additional tests, i.e. cytochrome oxidase test.

Figure 4.2-4 Phase III

1. Select colonies for antibiotic testing on basis of information derived from Phase II.

2. Use thin calcium alginate swab to carefully pick isolated colony from surface of primary isolation plate and spread on fresh bolld agar plate according to the following procedure:



3. Apply sensi-discs and incubate at 37°C for 18-24 hours.

4. Examine incubated plates for presence/absense and/or degree of inhibition. Check each plate carefully to determine if different colony types exist, i.e., mixed culture.

4.3 Assessment of Possible False Positives by Literature Search Coorelation

Determination of possible interfering organisms, with respect to the 10 selected organisms, is of critical importance to the reliability of the proposed system (I/D Key). Other bacteria of medical importance may exhibit identical reactions to the array of diagnostic tests; therefore, information should be evaluated as to the estimated frequency and the kinds of different micro-organisms which could lead to incorrect, presumptive identifications.

In preparing this section, text books which emphasized procedural methods, i.e., "How-To", etc. where consulted, rather than papers from scientific journals. The text books employed are listed in alphabetical order at the end of this section as Table 4.3-2.

In addition, one assumption was made: Normal preflight procedures will include extensive microbiological analysis of the crew together with strong emphasis on cleanliness and decontamination efforts with respect to the spacecraft interior. If defined procedures were systematically and vigorouly pursued, certain medically significant bacteria could be realistically ignored or eliminated from consideration as potential false positives, e.g., Bordetella pertussis or Listeria.

1. Streptococcus pyogenes

   Sensitivity of the S. aureus to the Taxo A disc and Neomycin disc, plus rather pronounced beta (clear) hemolyois, make this organism unique within this identification scheme (I/D Key). Other organisms which may cause hemolysis on Blood Agar include: Listeria, various corynebacteria, hemolytic Staphylococcus, E. coli, and Pseudomonas. However, none of these other possibilities have been reported to demonstrate sensitivity to the Taxo A disc; they are also unambiguously separable by Gram staining. The Lancefield Grouping, of which S. pyogenes is designated as

to Group A, lists 3 additional groups of medical significance, i.e., "C", "G", and "D" (found most frequently in urinary infections), which also exhibit beta hemolysis. Again, sensitivity to the Taxo A disc identifies S. pyogenes (Group A) from the others.

The genus Streptococcus represents an ubiquitous group of bacteria found frequently both in the on man and animals. Other commonly isolated strains of Streptococcus include S. faecalis (enterococcus) commonly isolated from feces or urine, exhibits beta homolysis and is classified under Lancefield Group D (above). S. mitis formerly S. viridans, demonstrates alpha (greening) hemolysis and is considered a normal comensal in the oral cavity of most subjects. This species is not likely to cause interference with the I/D Key in separating S. pyogenes.

Estimate: High probability (90-100%) of identifying S. pyogenes within this system.

2. Diplococcus penumoniae

Sensitivity to the Taxo P disc and alpha hemolysis are solid clues to identity. Interference may arise from Haemophilus influenzae which is also Taxo P sensitive and exhibits alpha hemolysis. Gram staining is necessary to separate the two: D. pnemoniae is Gram positive with oval or spherical forms typically in pairs and encapsulated. H. influenza stains a short, fat Gram negative rod.

Estimate: Other stains of Diplococcus not known; if Gram stain is performed to eliminate H. influenzae; There is high probability of positive identification (90-95%).

3. Haemophilus influenzae

Discussion above on D. pneumoniae is applicable. There are 15 spp. of H. influenzae for the genus Haemophilus, but only about 6 are medically important or apt to appear. The Haemophilus stains are fastidious in their nutrient requirements (require x + v factors for growth), and variable in their response to Taxo P discs. Alpha hemoysis may or may not be exhibited which may cause confusion with the alpha hemolytic S. mitis; This is augmented by variable sensitivity to the Taxo P disc. Gram staining will be needed to unequivocally separate these two. Catalase is not testable, but most Haemophilus strains are cytochrome-oxidase positive. Bordetella pertussis also exhibits similar reactions and requires blood-rich medium to effect isolation; however, the possibility of encountering B. pertussis in this particular situation (spacecraft) has to be considered remote. The problem then becomes one of recognizing Haemophilus strains which may or may not exhibit hemolysis or demonstrate sensitivity to Taxo P discs, primarily through Gram staining. Also, H. influenzae is sensitive to Neomycin.

Estimate: Possibility 60-70% of arriving at presumptive identification of H. influenzae.

4. Staphyloccus aureus

Staphylococcus is separable from Streptococcus primarily through the catalase test (Straph is positive). However, objective separation of Staph. aureus from S. epidermidis, S. saprophyticus and Micrococci spp. with the limited array of test available in the I/D Key is probably not possible. Testable biochemical differences exist; separations are also possible with anaerobic incubation, but neither are available within the I/D Key System. S. aureus does display a smooth, shiny, milky-white or yellow colony on BA, and after approximately 20 hours the colonies tend to exhibit a small ring of beta hemolysis around the periphery.

Gram stain characteristics between S. aureus and Micrococci can be significant: Micrcci normally display Gram positive spheres in pairs, fours or small clusters, with the cocci generally being more uniform in size. Straphyloccocci, on the other hand, tend to occur as Gram positive spheres in large clusters with the cells showing great variation both in size and stain-retaining power.

There is the possibility of confusion of S. aureus with Aerococci which can show variable reactions toward the catalase test (Aerococci can be feebly positive or frankly negative). Aerococci also demonstrate alpha hemolysis. However, the possibility of encountering Aerococcus is considered remote.

Estimate: The site of the sample will probably furnish the best indication for separation of S. aureus from S. Epidermidis. Separation of S. aureus from the Micrococci is dependent largely upon the operator's training, experience, and judgement. Identification of S. aureus: 75-85%

5. Candida albicans

Separation of Candida albicans from bacterial specimens by visual observation alone will require judgement. Candida can be isolated from almost any body site and generally exhibits a smooth, pasty colony on the BA. This colonial morphology is in contrast to other fungi which generally show a difuse, filamentous growth. The organism most likely to appear which can be confused with Candida is Geotrichosis candidum which can be isolated from a variety of infected material. G. candidum colonies are membranous, mealy, flat and cream to white in color; this color and morphology is similar to C. albicans. Gram staining is definitely needed to separate C. albicans from G. candidum: Under low power, Candida exhibits pseudomycelium with chlamydospores. G. candidum will exhibit rectangular arthrospores and true, braching hypae. C. albicans also exhibits complete resistance to all the antibiotic sensi-discs employed in this I/D Key.

Estimate: With a little experience, Candida albicans shoul be presumptively identified 90% of the time it occurs.

6. Shigella dysenteriae

S. dysenteriae is a member of the family Enterobacteriaciae, but is the only Shigella species which is catalase negative, making it a unique separation. The most likely organism which might be confused with S. dysenteriae is Actinobacillus (Glanders). However, Actinobacillus grows very slowly, and although it is a Gram negative rod, it tends to stain very irregularly and display a high incidence of pleomorphism.

On the other hand, S. dysenteriae displays strongly Gram negative, large fat rods. Actinobacillus is judged an unlikely candidate to be isolated. Separation of other strains of Shigella (catalase positive) are discussed in a following paragraph.

Estimate: Preumption identification of S. dysenteriae is considered 90-100%.

7. Pseudomonas aeruginosa

P. aeruginosa, if it is in sufficient numbers on the isolation plate, can often be recognized by a characteristic grape-like odor and a green pigment exhibited by the colonies on the agar. P. aeruginosa is catalase positive and Gram negative, displaying large, fat rods to long, slender, almost filamentous rods. Separation from the Enterobacteraciae is by the cytochrome-oxidose test which is negative for Enterobacteriaciae.

Estimate: Estimate 90-100% possibility for presumptive identification within I/D Key.

8. Enterobacter aerogenes

9. Proteres morganii

10. Salmonella typhi

The three organisms listed above are also members of the Enterobacteriaciae family. This represents a rather large division which is divided into

5 major groups, which in turn, encompass a minimum of 17 identifiable strains of enteric bacteria. Table 4.3-2 describes these subdivisions and the strains involved. In addition, Actinobacillus, Pasteurella, and Chromobacterium are possible interfering genera, although the probability of isolating these organisms under spaceflight conditions is judged unlikely. The major problem likes with the separation among the Enterobacteriaciae, even within the 5 major subdivisions. No test or procedure is available within the currently structured I/D Key that will objectively separate these groups. Expert bacteriologists might make venture educated guesses as to genera, based on the sample site, colonial morphology and gram stain cellular structure, but it is unreasonable to expect amateur microbiologists to attempt such subjective identifcations.

It is recommended that the Roche "Enterotube" be considered for inclusion within the diagnostic tests currently defined by the I/D Key. The device consists of an 8-chambered tube containing selected, sterile agar media. A sterile, protected rod, which runs down the middle of each chamber, is accessible through removable end-caps. To use, one end of the rod is touched to a single colony on an isolation plate, and the rod is withdrawn through the chambers, successively inoculating each chamber. The final chamber contains a positive control which assures inoculation. After incubation, color changes in each chamber are noted and the organism is identified by consulting a reaction chart and a table in which all possible permutations are displayed. (See Appendix, Section 6.5)

Estimate: Without additional test capability, there is virtually no possibility of presumptively identifying any of the Enterobacteriaciae.

TABLE 4.3-1

BIBLIOGRAPHY - SOURCES CONSULTED IN ASSESSMENT OF FALSE POSITIVES

Baily, W. R. & Scott, E. G: Diagnostic Microbiology. 2nd Edition, C. V. Mosby Co., St. Louis, 1966.

Bergey's Manual of Determination Bacteriology. 7th Edition, Edited by Breed, R.S., Murray, E.D.G., and Smith, N.R., The Williams & Wilkins Co., Baltimore, 1957.

Cowan. S.T. & Steel, K.J.: Manual for the Identification of Medical Bacteria. Cambridge University Press, 1966.

Gradwohl's Clinical Laboratory Methods and Diagnosis, Volume 1, 6th Edition. Edited by Frankel, S., Reitman, S., and Sonnenwirth, A.; C. V. Mosby Company, Saint Louis, 1963.

Pelczar, M. I.: Manual of Microbiologial Methods by the S.A.M. Committee on Bacteriologic Technic, McGraw-Hill Book Co., Inc., New York, London, Toronto, 1957.

Rohde, Paul A., Editor, BBL Manual of Products and Laboratory Procedures, 5th Edition, copyright 1958, 3rd reprinting 1970. BBL, Div. of Becton, Dickinson and Co., Cockeysville, Maryland 21030.

Schneierson, S. S.: Atlas of Diagnostic Microbiology, Published by Abbott Laboratories, North Chicago, Ill., 1965.

Skerman, V.B.D., A Guide to the Identification of the Genera of Bacteria, 2nd Edition, The Williams & Wilkins Co., Baltimore, 1967.

Table 4.3-2

*Group Differentiation of Gram-Negative Organisms (Enterobacteriaciae)

	Group	Genera	Strains
1.	Escherichia Shigella	Escherichia Shigella	
2.	Proteus Providencia	Proteus	Vulgarie Mirabillis Morgonii Rettgeri
		Providencia	
3.	Klebsiella Enterobacter Seratia	Klebsiella Enterobacter	Aerogenes Aerogenes Hafniae Liquefaciens
		Serratia	
4.	Salmonella Arizona Citrobacter	Salmonella Arizona Citrobacter	
5.	Edwardsiella	Edwardsiella	

*Roche Diagnostics, Division of Hoffman-LaRoche , Inc.
Nutley, New Jersey 07001

4.4 RESULTS OF LIVE SUBJECT TESTING USING I/D KEY

Evaluation of the I/D Key can only be accomplished by applying those tests and procedures to actual subjects. The question of how many subjects and what sample sites is addressed in the Statement of Work: A total of 10 subjects will be sampled from each of 3 sites, i.e., throat, nose, and skin (forearm), for a total of 30 samples to be subjected to analysis (presumptive identification) according to the scheme derived from the I/D Key. Omission of fecal samples obviated the possibility of uncovering 4 of the selected organisms (the Enterobacteriaciae), i.e., Proteus morganii, Shigella dysenteriae, Salmonella typhi, and Enterobacter aerogenes. Although the total number of subjects is small, statistically, it was felt that sufficient information could be extracted to derive meaningful and useful information about the Key.

4.4.1 TEST PROTOCOL

During actual testing, thirteen subjects were selected at random from the GE-Valley Forge STC population. Attempts were made, through the in-house Health Service, to obtain subjects suffering from obvious, upper-respiratory infections. Due probably to the time of the year (late summer), and the relatively short period devoted to actual testing (3 weeks), it was not possible to find such subjects, other than those suffering from obvious allergic-type reactions.

Three Caligswabs[3] (bulky) were used to collect samples from each subject; soft pallet area in the throat (dry swab); nares (swab moistened in sterile Nutrient broth); and skin of forearm (moistened swab). Each swab was streaked on the upper quarter of a Blood Agar (BA) isolation plate. Using 3 additional, sterile swabs, a "plate dilution" was effected. Immediately therafter, a Taxo A, a Neomycin (30 mcg) disc, and a Taxo P disc were placed over the area of the original streak

[3] See Footnote #2

(heaviest concentration). This procedure is identical to that outlined in Figure 4.2-2, 3, 4: Simplified Procedures for Use of I/D Key. Incubation was at 37°C for 22-24 hours.

The information resulting from each swab sample/BA plate was recorded on a form devised for the testing. An example of this form is shown as Figure 4.5-1. The raw data collected in reproduced in the Appendix, Section 6.4.

4.4.2 RESULTS OF LIVE SUBJECT TESTING

Results of the testing of 13 subject from 3 sample sites are summarized on Table 4.5-1.

As shown in the Table, the two most numerous genera isolated were staphylococcus and non-pathogenic streptococcus. Differentiation between staph and strep was easily accomplished by use of the catalase test (staph is calalase positive). However, differentiation between staph and micrococcus spp. or strep and areococcus spp. was not possible, nor was accurate separation possible within the strep. spp. itself except possibly for S. mitis which was identifiable by virtue of the alpha homolysis and non-sensitivity to Taxo-A discs. S. mitis was identified in every throat culture of the 13 subjects.

Candida albicans was tentatively identified from the Gram stain microscopic examination and comparison with the photo-micrographs which accompany the I/D Key.

Identification of the Corynebacterium spp. was accomplished on the basis of colonial morphology and Gram stain examination which displayed typical palisading arrangements among the club-shaped, intensely Gram positive rods. This differentiation is not a part of the system I/D capability.

There was a total of 4 organisms which were essentially untypable through employment of the diagnostic tests available with the I/D Key. No other attempt was made to identify these isolates by use of standard sugar firmentation tests, typing Sera, etc.

In addition, the following general observations can be made concerning the live subject testing:

1. The calcium Alginate Swabs, both the bulky and thin varieties, are excellent substitutes for the microbiologists's loop.

2. The swabs become less than optimum when good physical separation of the different colony types has not occurred as a reoccurrence is the result of variable which are essentially uncontrollable, and therefore, not amendable to corrective suggestions.

3. The technique suggested by the I/D Key is simple and straight-foreward. The primary emphasis during the construction of this key was to provide for a series of bionomial steps which would led, eventually, to a logicial, unabmiguous result, i.e., a presumptive identification. Experience to date indicates that this circumstance cannot be expressed in every instance.

4. The deficiencies alluded to above can be overcome to some extent by sufficient skill on the part of the operator, i.e., training; or by adding one or two additional diagnostic tests to the basic armamentarium; optimally, both.

5. The catalase test is one of the more useful tests employed. It appears that with some strains likely to be encountered, e.g., Micrococcus spp., Aerococcus spp., the amount of cellular material gathered for this test may be critical. The difference between a true positive and a false negative may be dependent upon a critical mass necessary to confirm by visual examination the gas bubbles produced by the addition of the hydrogen peroxide.

It was concluded that the live subject testing was not structured in sufficient depth to answer statistically the degree of confidence with which the 10 selected organisms could be identified presumptively during random testing. Never the less, the exercise did provide invaluable information on the technique, and their applicability by non-professional micro-biologists. Such a preliminary test would have been required before embarking on a large scale testing exercise.

MICROBIAL IDENTIFICATION

CODE NUMBER: _____

SUBJECT NAME: _____

SITE: _____

SWAB: MOIST - _____ DRY - _____

MEDIUM - _____

DATE: _____ TIME: _____

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: _____

AROMA (IF PRESENT)

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM.		COLONY CHARACTERISTICS					
				B	C	D	E	F	G
		CATALASE	CYTOOXIDASE	FORM	COLOR	EDGE	ELEVATION	OPTICAL CHAR.	HEMOL.

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER						PRESUMPTIVE I/D
	AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

FIGURE 4.5-1

TABLE 4.5-1

SUMMARY OF LIVE SUBJECT TESTING USING I/D KEY

ORGANISM-PRESUMPTIVE IDENTIFICATION	SAMPLE-SITES THROAT	NARES	SKIN
Strep/Aerococcus	29	5	5
Staph/Micrococcus	8	18	11
Candida	0	1	0
Other			
Corynebacterium	1	4	2
Unknown	2	4	0
Total	40	30	18

5.0 CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Results of the experimental laboratory work and literature analysis on the simplified identification scheme indicate adoption of the following conclusions:

- o The test and procedures defined for the study are satisfactory for implementation of the I/D Key concept for 4 of the selected organisms (Enterobacteriaciae).
- o The possibility of the occurrence of false positives is minimal with the significant exception of the 4 organisms cited above.
- o The basic design concept of the simplified presumptive identification is sound.
- o Evaluaction of the I/D Key and procedures with limited, live subject testing has demonstrated the value and applicability of the procedural tools and techniques.

RECOMMENDATIONS

Mindful of the preceeding conclusions, the following recommendations are made:

- o Insertion of the Roche "Enterotube" into the array of diagnostic tools and procedures to effect separation and presumptive identification of the family, Enterobacteriaciae (see Appendix, Section 6.5).
- o Use of some modest form of optical magnification (2x-5x) to aid the onboard operator to describe and define more accurately the gross morphological characteristics for real time ground information.
- o Emphasis on microscopic training and technique as a critical skill in implementating the I/D Key.
- o A study of the critical amount of cellular material necessary to perform the Catalase test, i.e., eliminate possibility of false negatives.
- o Investigate a statistically validated field trial for a more extensive evaluation of the I/D Key and procedures.

SECTION 6

APPENDIX - PROTOCOLS AND RAW DATA

6.1 REHYDRATION OF STOCK CULTURES FROM ATCC

Bacterial and fungal cultures from the Americal Tupe Culture Collection (ATCC) are routinely sent through the mail in the lyophilized state. The dried material is encased in a glass vial protected by a cotton plug. This vial is encased in a larger flame-sealed glass capsule with asbestos wadding between the cotton plugged inner vial and the larger, protective vial. The entire assembly is wraped in cotton wadding and packed inside a double shipping container. Thus, the likelihood of breakage is slight and, if there is, the danger of possible infection is greatly reduced.

To open the vial and rehydrate the culture for experimental use, the following equipment is suggested:

1. Bunsen-type flame
2. Container of water (sterile condition not required) and pipette
3. Tubes containing sterile bacteriological broth, i.e., Nutrient broth, Tryp-Soy broth, etc. Amount of material is dependent upon number of cultures to be rehydrated - 0.5 ml/culture.
4. Sterile pipettes
5. Sterile foreceps
6. Standard bacteriologist's loop
7. Tubes, poured plates, agar slants, etc. of prepared media - depending on micro-organisms to be cultured.

Procedure

1. Carefully heat the flame-sealed end of the outer protective vial in the Bunsen flame. The asbestos wadding protects the smaller vial containing the lyophilized culture from heat.

2. Allow a few drops of water to dribble on the heated end of the glass. This will cause the glass to crack and allow access to the inner vial.

3. With the sterile foreceps, carefully remove the inner vial.

4. Loosen the cotton plug in the culture vial.

5. Remove the cotton plug and introduce 0.5 ml of appropriate sterile broth (sterile, distilled water may also be employed) and allow dried material to rehydrate. The tip of the sterile pipette used to introduce the broth may be used to mix the solution. The time required to solubilize the dried material varies with the nature of the organism, the original suspension-liquid, and the efficiency of the lyophilization process.

6. Distribute portions of the rehydrated material into appropriate culture tubes, etc. for incubation.

6.2 ROUTINE PLATE COUNT ASSAY OF STOCK CULTURES

It was considered essential that each test organism demonstrate a stable, viable count following constant incubation conditions, i.e., 18 hours at 37°C before subjecting them to any of the defined tests or procedures. Consequently, plate count assays were performed on successive 18 hour cultures of each organism, except H. influenzae[4], until stable titers were obtained. It was considered that this condition represented a state of "metabolic maturity" for each micro-organism, and thus would exhibit reproduceable reactions to the standard tests/ procedures for the I/D Key.

The following procedures were pursued during standard pour-plate determinations:

1. Screw-cap culture tubes (20 x 150 mm) were employed as growth tubes, containing 9 ml each of either Typticase-Soys broth or Nutrient broth (see Table 3.1-1 in text).

2. Each tube was inoculated with one drop of the previous 18 hour culture which had been stored at 4°C until needed.

3. Tubes were incubated at 37°C for 18 hours with screw caps loose, i.e., not screwed down.

4. Mixing of each culture tube was accomplished in the following steps:

    a. Agitation for 30 seconds in a mechanical mixer, e.g., Vortex Jr.

    b. Additional agitation for 2 minutes in a Bronson "Sonigen" ultra-sonic generator, filled to predetermined mark with a solution of 0.3% Tween 80 in distilled water. The tube was inserted in the bath so that the level of fluid in the tube equaled the level in the bath. The generator was tuned by ear to the highest discernable pitch.

5. Each tube was serially diluted in 10-fold steps using 9 ml blanks of either Tryp-Soy or Nutrient broth. Mechanical agitation was employed between each dilution.

6. The appropriate dilution tube was used to provide triplicate samples of 0.1 ml each, i.e., 10^5 diln. $\xrightarrow{0.1\ ml}$ 10^6 plate. Three dilutions per sample were plated.

[4] H. influenzae was cultured by successive transfers on Blood Agar plates.

7. Melted agar, held at 45°C in a water bath, was poured into each of the sample plates and swirled to mix the agar medium. The same nutrient agar base was employed for the plate medium as that used for 18 hour growth medium and for the dilution fluid.

8. Plates were allowed to solidify, inverted, and placed at 37°C for 48 hours. Plates were counted with the aid of a New Brunswick colony counter.

6.3 DIAGNOSTIC TEST PROCEDURES

Taxo Discs

Taxo A and P discs were employed in connection with the Neomycin (30 mcg) disc. Each disc was placed in the upper quarter of the isolation plate (Blood Agar Plate) in the area of the initial spreading of the sample (heaviest concentration). Discs were always placed about 1 disc-diameter apart in order to provide sufficient separation of possible areas of inhibition, and to allow space for individual concentration gradients resulting from each disc. No effort was made to consider possible effects of disc placement during a zero gravity environment, although the problem of dispensing these disce during weightless operation appears to present no serious technical problems.

Antibiotic Sensi-Discs

The discussion above is applicable to the sensi-discs. The application of the discs singly or simultaneously during zero gravity operations is a detail which can be addressed at a latter date.

Catalase Test

Two methods were used to test for the presence or absence of Catalase: filter paper technique and test tube assay.

1. Filter Paper

   A thin "Calgiswab" was used to obtain cellular material from an isolated colony on the Blood Agar isolation plate. The swab was then rubbed across the surface of a strip of filter paper (approximately 10 x 75 mm). Either a syringe or a pipette was used to apply small amounts (0.2 - 0.4 ml) of 3% hydrogen peroxide (H_2O_2) directly to the filter paper. The immediate expression of bubbles on the surface of the filter paper indicated the

presence of Catalase. Performed in this manner, the test is directly applicable to zero gravity operation.

Comment - Continued experience with the test suggested that absolute discernment of the presence or absence of bubbles was not always possible. Indications were that the amount of cellular material deposited on the filter paper was critical. The Catalase test is an extremely useful test, particularly in the separation of Staph and Strep colonies. Therefore, a modest study to define, within some acceptable limits, the amount of cellular material required for reliable test results, i.e., production of easily visible bubbles, is indicated.

2. Test Tube Assay

In this variation, the "Calgiswab" containing the sample was inserted directly into a test tube (13 mm) containing approximately 0.5 ml of 3% H_2O_2. The expression of small bubbles from the vicinity of the cellular material was easily visible through the test tube. Some equipment modification would be necessary to employ this technique during zero gravity operation.

Comment - Care should be taken in this assay to differentiate between air bubbles trapped within the fibers of the swab and those bubbles which issue as a result of the catalase reaction. The former tend to be larger and few in number, while the latter are very small in size and quite rapidly expressed.

Cytochrome - Oxidase Test (Patho-Tec Strip)

Cellular material was obtained on a "Calgiswab" in the same manner as defined for the Catalase test and applied to the reagent end of the Patho-Tec Strip. With a

positive test, i.e., cytochrome-oxidase present, the reagent zones turn a bright blue color within 30 seconds; a negative result exhibits no change in color. Weakly positive reactions can occur where a pale blue color will develop. Addition of a few drops of water to the Patho-Tec Strip makes no discernable difference to the final results.

Gram Stain Technique

The Gram stain procedure employed in this study followed the technique as defined by the Hucker modification[5]. The procedure is as follows:

o Gentian Violet Solution	1 minute
o Gram's Iodine	1.5 minutes
o 95% Ethyl Alcohol	5-8 seconds
o Safranin (1% aqueous)	1 minute

Speciments for the Gram stain procedure were derived from pure cultures incubated for 24 hours on Blood Agar isolation plates. "Calgiswabs" were used to transfer cellular material from a single colony to standard glass slides containing a drop of sterile water. The dispersed samples were fixed with gentle heat (40-45°C) for a minimum of 30 minutes before initiating the staining sequence. Cover slips were permanently affixed on each slide using "Permount"[6].

[5]New York Agricultural Experiment Station Technical Bulletin, 1927, page 128.

[6]Obtained from Fisher Scientific Company, Cat. No. So-P-15.

6.4 RAW DATA FROM LIVE SUBJECT TESTING

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MICROBIAL IDENTIFICATION

CODE NUMBER: D-W.

SUBJECT NAME: Dixie Wright

SITE: 2 - Throat

SWAB: MOIST - _____ DRY - ✓

MEDIUM - —

DATE: 8/16/91 TIME: 1415

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-(24) HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

~~DESCRIPTION~~:

Neo 30 - Inhib. of white colony, not α hemol. colonies

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a @	+Ve staphylococcus	+ve	N.D	irregular	2	1	3	2	1-3
* 1-b	+ve staphylococcus	+ve ±	N.D	White	2	1	3	1	2

ck/Path OK

* No isolated colonies available - may be mixed culture (8/17/91)

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							Diplococcus/Strep
1-b							micrococcus

@ very pleomorphic, short/long chains of tight form, short/fat forms, clumps, etc.

@ mostly small, fairly uniform size

43

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: D.W

SUBJECT NAME: Dave Wright

SITE: 2 - Nares

SWAB: MOIST - _____ DRY - ✓

MEDIUM - N13

DATE: 8/10/71 TIME: 1415

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

~~β hemol. inh.~~ No

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

DESCRIPTION:

Neo 30 - complete inhib.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTO-OXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a	g+c clumps & tetrads	Pos.	ND	white	2	1	3	1	1-4
2-b	g+c dispersed	Pos	ND	white	2	1	3	1	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
-2-a							Staph
2-b							Staph/micrococcus

① Uniform size, some short chains, singles

② In all configurations, 1, 2, 4, short chains, small clumps

MICROBIAL IDENTIFICATION

CODE NUMBER: D.W.
SUBJECT NAME: Dave Wright
SITE: 3. Skin (forearm)
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/16/71 TIME: 1415

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____

TAXO P - PRESENT _____ ABSENT _____ / Too Few To Tell

NUMBER OF APPARENT COLONY TYPES: 1

AROMA (IF PRESENT)

DESCRIPTION:

New 30 - TFTT

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3-a	gtc singles clumps ✓	Pos	ND	white	2	1	3	1	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3-a							Staph

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: G-F.
SUBJECT NAME: Gordon Fogal
SITE: 1- Throat
SWAB: MOIST - _____ DRY - ✓
MEDIUM - —
DATE: 8/12/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT)

~~DESCRIPTION:~~
Neo 30 & Inhib. 3 of hemol. colony.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a ①	g+c small curvy	Neg	ND	colorless	2	1	3	2	1-3
1-b ②	g+c singles chains	Neg	ND	white	2	1	3	2	2
1-c ③	g+c singles doubles	Pos+	ND	Ivory	2	1	4	2	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							(Diplococcus) Strep [illegible]
1-b							Staph [illegible] Staph/micrococcus
1-c							

① many single, double forms. Light config.
② small clumps, over decolorized (gram-neg). irreg. size
③ strongly g+, irreg-size, short chains. generally small in size.

MICROBIAL IDENTIFICATION

CODE NUMBER: G ~~FA~~
SUBJECT NAME: Borden Fogal
SITE: 2-NCHS
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/12/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

~~DESCRIPTION:~~

New 30 - complete inhib

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a ①	g+c ~~singles~~ clumps	Pos	ND	circular	2	1	3	1	2
2-b ②	mixed cultures?	Pos	ND	rhizoid	1	1	3	2	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							Staph/micrococcus coryneb.
2-b-1							
-2							2.

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

① strongly g+, large, uniform cocci.

② a) large, club-shaped, strongly g+ rod, irreg. staining

b) slender g- rod, some w vacuole-like body near end of cell, variable length, some bulbous-shaped

MICROBIAL IDENTIFICATION

CODE NUMBER: G. F.
SUBJECT NAME: Gordon Fogal
SITE: 3. Skin (forearm)
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/12/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-~~24~~ HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____

TAXO P - PRESENT _____ ABSENT _____

Too Few To tell

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

~~DESCRIPTION~~:

NLO - TFTT

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3-a ①	g+c clumps	Pos	ND	white	2	1	3	1	2
3-b ②	g+c mostly doubles	Neg	ND	grey	1	1	3	1	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3-a							Staph
3-b							Strep/Aerococcus

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

① Strongly g+, larger, uniform size, singles, doubles, tri's, 4's, etc. few short chains.

② Strongly g+, irreg size, some singles, short chains

MICROBIAL IDENTIFICATION

CODE NUMBER: P.M.
SUBJECT NAME: Peter Marchetta
SITE: 1 - Throat
SWAB: MOIST - _____ DRY - ✓
MEDIUM - _____
DATE: 8/17/71 TIME: 1340

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT)

~~DESCRIPTION:~~

new 30 - ~~at~~ hemol. colonies not inhibited

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a (1)	+ diplococcus	Neg	ND	Convex	1-2	1	3	2	1-3
* 1-b (2)	g+c mostly streptococci	Neg	ND	yellow white	2	1	3	1	2
* 1-c (3)	g+c as above mixed ~~culture~~ smear	Neg	ND	Ivory	6	6	5	1	2

* Colonies not isolated - may be mixed sample/smear.

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							diplococcus/strep
1-b							strep/~~staph~~ enterococcus
1-c							strep

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

(1) Small, coccoid shape envelope present
(2) Some small clusters, slightly ~~over~~ decolorized, variable size, some large, strongly g+c absent - mixed culture ?
(3) Evidence of ~~one~~ decolorization, short chains of bacilli, some branches, small, variable cocci - ~~1 c~~ strongly g+ cocci

MICROBIAL IDENTIFICATION

CODE NUMBER: P-M

SUBJECT NAME: Peter Marchetta

SITE: 2- Nares

SWAB: MOIST - ✓ DRY - _____

MEDIUM - NB

DATE: 8/12/71 TIME: 1340

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: 1 - (w 2 color forms)

AROMA (IF PRESENT)

~~DESCRIPTION:~~

Neo 30 - complete inhib.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a ①	g+c large, uniform	Pos	ND	yellow	2	1	3	1	2
2-b ②	g+c large, uniform	Pos	ND	white	2	1	3	1	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							staph
2-b							staph

① mostly singles & clumps, some short chains

② As above

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: P-M

SUBJECT NAME: Peter Marchetta

SITE: 3- Skin (forearm)

SWAB: MOIST - ✓ DRY - _____

MEDIUM - NB

DATE: 8/17/71 TIME: 1340

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____

TAXO P - PRESENT _____ ABSENT _____

Too few To Tell

NUMBER OF APPARENT COLONY TYPES: 2 (w 2 color forms)

AROMA (IF PRESENT)

DESCRIPTION:

Neo -30 - T F T T

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3-a (3)	g+ rod	Pos	ND	gray	4	4	3	1	2
3-b (2)	g+c large, uniform	Pos	ND	yellow	2	1	3	1	2
3-c (1)	g+c large, clumps	Pos	ND	white	2	1	3	1	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3-a							coryneform
3-b							staph
3-c							staph

(1) large, strongly g+ rod, much intense pigmentation

(2) mainly g+c, uniform, singles & small clumps

(3) large clumps, strongly g+, almost all in clumps
large cocci

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: JC
SUBJECT NAME: John Carey
SITE: 1 - Throat
SWAB: MOIST - _____ DRY - ✓
MEDIUM - —
DATE: 8/16/71 TIME: 1400

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT)

~~DESCRIPTION~~:

Neo 30 - No inhibition of a hemol. colonies

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Probably same colonies (48 hr. observation)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a ②	g+c small diplococci	Neg	ND	colorless	2	1	3	2	1-3
1-b ③	g+c clumps of c. & short chains	Neg	ND	white	2	1	3	1	2
1-c ①	g+c singles, clumps	Neg	ND	grey	4	4	5	1	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							Diplo / strep
1-b							strep / [illegible]
1-c							strep

① Some small chains, very small cocci, some diplo forms: difficult to distinguish
② strongly gram var. small cocci, irreg in shape
③ large, strongly gt cocci, some variability in size

MICROBIAL IDENTIFICATION

CODE NUMBER: JC
SUBJECT NAME: John Cary
SITE: 2 - Nares
SWAB: MOIST - _____ DRY - _____
MEDIUM - NB
DATE: 5/16/71 TIME: 1400

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT ✓

} β-hemolytic colony inhibition

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

DESCRIPTION:
New 3 - complete inhib.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a①	g+c clumps impinging size	Pos	ND	white	2	1	3	1	1-4
2-b②	g+c large clumps	Pos	ND	white	2	1	3	1	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							staph
2-b							staph/micrococcus

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

① large cocci strongly g+

MICROBIAL IDENTIFICATION

CODE NUMBER: J. C.
SUBJECT NAME: John Cary
SITE: 3 - Skin (forearm)
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/16/71 TIME: 1400

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24) HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____ Too Few To Tell

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: 1

AROMA (IF PRESENT)

DESCRIPTION:

New 30 - TFTT

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3-a	g+c clumps small	Pos	ND	white	2	1	3	1	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3-a							Staph

① Disposed organism strongly g+

MICROBIAL IDENTIFICATION (1)

CODE NUMBER: TC
SUBJECT NAME: Therese Cherry
SITE: 2 - Throat
SWAB: MOIST - _____ DRY - ✓
MEDIUM - _____
DATE: 8/11/71 TIME: 1345

PRIMARY CULTURE INFORMATION
RESULTS ON BLOOD AGAR (18-24 HOURS)
TAXO DISC REACTION:
TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT ✓
NUMBER OF APPARENT COLONY TYPES: 3
AROMA (IF PRESENT) N/A
~~DESCRIPTION~~:
Neomycin 30 Inhibition of white colonies but not colorless/hemolytic (alpha) ones colonies
OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

g+ diplococci in chains, some pairs large; variable staining chains; not like gonococci

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
SC 1 a	(1) g+c ~~streptococci~~ diplococci	Neg	ND	~~white~~	2	1	3	2	1-3
SC 1 b	(2) g+c uniform size	Neg	ND	white	2	1	3	1	1-4?
SC 1 c	(3) g+c singles and doubles	Neg	ND	grey	2	1	3	1	2

Check 48 hrs.

(2)

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D

See Lost Page #4

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

55

(3) Over decolorized, but definitely g+ — no chains or clumps, not organisms or doubles - make some look like short rods
(2) non-uniform formation - having some cells staining g+ cocci - probably over de-colorized i.e. g+c not g- as many cells demonstrate

SC = Subculture

(2)

MICROBIAL IDENTIFICATION

CODE NUMBER: TC -2
SUBJECT NAME: Therese Cherry
SITE: 2 - Nares
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/11/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

~~DESCRIPTION:~~ Neo -30 : complete inhibition about disc

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a	g+c mostly in clusters & singles	P 3	ND	white	2	1	3	1	1-4
2-b (2)	g+r incompl. stain pale staining	Neg		white	1	1	3	1	2

Staph. 4 Plus.

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							Staph
2-b							corynebacterium

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

(2) club shaped, strongly g+, great variability in size

MICROBIAL IDENTIFICATION

CODE NUMBER: TC (3)
SUBJECT NAME: Therese Curry
SITE: 3 Skin (forearm)
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/11/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____
TAXO P - PRESENT _____ ABSENT _____ } too few colonies to tell

NUMBER OF APPARENT COLONY TYPES: 1

AROMA (IF PRESENT) N/A

DESCRIPTION:

NB 30 — Too few colonies to tell

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3a (1)	g+r & g-r (2)	Pos		white	2	1	3	1	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3a1							coryneb. sp
3a2							Staph

(1) Mixed culture? (2) large, strongly g+ rod in variable staining and club shaped appearance.
(2) slender, g- rod showing possible palisading - possibly indicates difficulties in ~~dec~~ rate of decolorization!

1-a — Very sparse growth at 24 hrs. - only 24hrs. colonies visible - appears to be pure culture at 24 hrs. - No hemolysis

18hrs. - No change

MICROBIAL IDENTIFICATION

CODE NUMBER: TC - 2 (1)

SUBJECT NAME: Therese Cherny

SITE: Subculture 1-a 1-c 1-b 3-a

SWAB: MOIST - _____ DRY - _____

MEDIUM - _____

DATE: 8/16/71 TIME: 1600

PRIMARY CULTURE INFORMATION - Re culture

RESULTS ON BLOOD AGAR (18-(24) HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: _____

AROMA (IF PRESENT)

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a (1)	g+c single & doubles	Pos ±	ND	colorless	2	1	3	2	2
1-b₁ large (2)	g+c singles & chains	Neg	ND	white	2	1	3	1	2
1-b₂ small (3)	g+c chains	Neg	ND	white	2	1	3	1	2
1-c (4)	g+c singles & doubles	Neg	ND	white	2	1	3	1	2
3-a₁ (5)	g+R fat mostly short,	Pos ±		white	2	1	3	1	2
3-a₂ (6)	g+c singles gram var.	Neg?		gray	4	1	3	1	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							micrococcus
1-b₁							Strep
1-b₂							Strep
1-c							Strep

1-b - Heavy growth; appears to be a gray/blue pigment present in area of heaviest inoculum. large, milky, shiny colonies and small, milky colonies difficult to say if 2 types or 1 at 24 hrs. No hemolysis

18hrs - Probably pure culture - degree of crowding makes difference in colonial appearance. Evidence of satellitism?

① some short chains, strongly g+, singe uniform, mostly in doubles
② large, uniform size, some short chains
③ Strongly gram, rods, fairly uniform size
④ large, strongly gram + some short chains
⑤ some club shape - some slight polar body
⑥ Contam - strongly g+ short fat rods

MICROBIAL IDENTIFICATION

CODE NUMBER: JL

SUBJECT NAME: Jane Laycock

SITE: 1 - Throat

SWAB: MOIST - _____ DRY - ✓

MEDIUM - _____

DATE: 8/4/71 TIME: 1410

PRIMARY CULTURE INFORMATION ~~Culture~~

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

~~DESCRIPTION~~:

New Inhibition only ? white gram-hemolytic ~~staphylococcus~~

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

white colonies few in number - not isolated for easy access

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a	① g+ rod & diplococci ②	neg	ND	colorless	2	1	3	2	1-3
1-b	② ± g+ short chains, c.3, etc. ± 4's	Neg?		white	2	1	4	1	2
48 hr.									
1-c	③ g+ mostly clumps ~~some singles~~			yellow white	4	3	5	1	2

Grow together

* May be mixed culture - no isolated colonies available

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
							Lost Page - 4
							See

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

① Very difficult to determine between rod & diplococci - Strongly g+; some suggestion of palisading and small, club-shape appearance, possible variable staining ~~pattern~~

② Great variability in size - some ovoid - ? contamination / mixed culture

③ ~~uniform size~~ - no evidence / mixed culture

MICROBIAL IDENTIFICATION

(1)

CODE NUMBER: JL ~~Jane Haycock~~

SUBJECT NAME: Jane Haycock

SITE: 2- Nares

SWAB: MOIST - ✓ DRY - _____

MEDIUM - NB

DATE: 8/11/71 TIME: 1410

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) N/A

~~DESCRIPTION~~:

New 3c - Complete inhibition

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a (1)	G+C mostly clusters some singles	POS	ND	white	2	1	3	1	1-4
2-b (2)	G+C clusters and singles	POS	ND	white ivory	2	1	3	1	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I.D
2-a							Staph
2-b							Staph/micrococcus

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

(1) uniform size
(2) Uniform Size

MICROBIAL IDENTIFICATION

CODE NUMBER: JL

SUBJECT NAME: Jane Laycock

SITE: 3 - Skin (forearm)

SWAB: MOIST - ✓ DRY - _____

MEDIUM - NB

DATE: 8/11/71 TIME: 1410

(3)

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____ N/A - 24 hr.

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: _____

AROMA (IF PRESENT) N/A

~~DESCRIPTION:~~ NEW 30 - N/A 24 hrs.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

No apparent growth in 24 hr.

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
—									

Check 48 hrs. No apparent growth

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

24hr. 1-a Pure culture at 24 hrs. good growth of large circular colonies. colonies showing strong α hemol.

48hrs. No change

MICROBIAL IDENTIFICATION

(4)

CODE NUMBER: J. L. - 7 - Resultant

SUBJECT NAME: Jane Laycock

SITE: Subculture 1-a, 1-b

SWAB: MOIST - _____ DRY - _____

MEDIUM - _____

DATE: 8/16/71 TIME: 1600

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: _____

AROMA (IF PRESENT)

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a	g+c diplococcus	Neg	ND	colorless	2	1	3	1	1-3
* 1-b, (2)	g+ diplococci	Neg	ND	Grey	2	1	3	1	1-3
1-b2 (3)	g+R Var. Staining	Pos ±	ND	colorless	1	4	3	2	2

* No isolated colonies available - probably mixed smear on Gram stain

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							diplococcus/strep
1-b-1							diplococcus/strep
1b 2							coryneb variation

(1) Some short chains, partially de-colorized, some pleomorphic forms

(2) Some short chains & diplo form

(3) Pleomorphic forms - club shape, some long slender, others short fat, evidence of palisading

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

1-b Mixed culture @ 24 hrs. (1) large, circular, opaque 24hrs. colonies w strong α hemol. mixed w other (2) punctiform colorless colonies in great abundance. no evidence of hemol.

48 hrs - No change

MICROBIAL IDENTIFICATION

CODE NUMBER: FR

SUBJECT NAME: Fred Rudek

SITE: I Throat

SWAB: MOIST - _____ DRY - ✓

MEDIUM - _____

DATE: 8/11/71 TIME: 1330

(1)

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION: type c

TAXO A - PRESENT _____ ABSENT no effect vs. a & b

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3-4 Some apparent colony types present in numbers and not isolated for sampling

AROMA (IF PRESENT)

DESCRIPTION:

Neo 30 - inhib. vs. a & c

Type d in very small numbers - Too few To tell.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a	(1) g+c many singles & 2's, 3's, 4's	Neg	ND	white	2	1	3	1	2
SC 1-b	(2) g+c chains	Neg	ND	colorless	2	1	3	2	1-3
SC 1-c	(3) g+c singles & doubles	Neg	ND	white	2	1	5	3	2
1-d	g+c clusters & uniform size	Pos	ND	irregular white	2	1	4	1	2

Numerous.

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							Strep (suspect)
1-d							Staph

(1) over decolorization; some short chains, fairly uniform size

(2) Very irregular staining, variable size, many doubles, difficult stain to interpret

(3) Sparse smear, no discernable clumps

SC = Subculture

MICROBIAL IDENTIFICATION

CODE NUMBER: FR
SUBJECT NAME: Fred Rudek
SITE: 2- Nares
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/11/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) N/A

~~DESCRIPTION:~~
Neo:30 - complete inhib

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a	(1) g+ var C Staphylo	Pos.	ND	white	2	1	3	1	1-4
2-b	(2) g+c clusters	Pos	ND	white	2	1	3	1	2

chief author

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							Staph
2-b							Staph/micrococcus

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

(1) occur mostly in packets, few singles
(2) Uniform Size

MICROBIAL IDENTIFICATION

CODE NUMBER: FR
SUBJECT NAME: Fred Rudek
SITE: 3 Skin (forearm)
SWAB: MOIST - C DRY - _____
MEDIUM - NB
DATE: 8/11/71 TIME: 1330

(3)

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____
TAXO P - PRESENT _____ ABSENT _____

Too few colonies to tell

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) N/A

~~DESCRIPTION:~~

New 3a - TFTT

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3-a (1)	gtc clusters	Pos	ND	white	2	1	3	1	2 t 4/5 hr check
3-b (2)	gtc clusters, 2s, 4/5	Pos	ND	yellow	2	1	3	1	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3-a							Staph
3-b							staph/micrococcus

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(1) small size (in regard to 2 b also)

(2) Great variability in size & shape. Some gram variability

24hrs. 1-b Mixed culture ① shiny, translucent colonies - overwhelming predominant in no hemol. ② small, irregular shiny colonies, widely dispersed showing α hemol ③ 2 large, irreg ivory colony probably chance contaminant

48hrs. 1-b - #1 above shows definite α hemol. #2 may be larger forms of #1 which managed to metabolize faster or different species also causing α hemol.

MICROBIAL IDENTIFICATION

(9)

CODE NUMBER: 1R-2

SUBJECT NAME: Fred Rudek

SITE: Subculture 1-b 1-c

SWAB: MOIST - _____ DRY - _____

MEDIUM - _____

DATE: 8/16/71 TIME: 1600

PRIMARY CULTURE INFORMATION Reculture

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT _____

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: _____

AROMA (IF PRESENT)

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-b, ①	g+c mostly singles & 2's - short chains	Neg	ND	colorless	1	1	2	2	2 (1-3)
1-b2 ②	g- r pleomorphic	Neg	ND	colorless	2	1	3	2	1-3
1-b3 ②	g+c singles and [illegible]	Pos	ND	white	4	1	3	1	2
1-C ③	g+c singles, doubles	Neg	ND	white	2	1	3	1	2

(1) many in ovoid shape in diplo form, some appear as g+ rods, short, in short chains

Singles g+c predominate

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-b1							staph/diplococcus
1-b2							2?
1-b3							staph/micrococcus
1-C							micrococcus

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

1-C 24hrs. Difficult plate to read at 24 hrs. Large, irreg and smooth round ivory colonies w small, shiny, irreg. white colonies - inhibited versions of large due to crowding or 2 distinct varieties?

48hrs. - Umbonate colonies quite similar on periphery ~~[illegible]~~ smear - crowded portion has smooth, opaque colonies

② Somewhat gram variable, bacillus form predominates

② Some forms long, slender g-r, others fairly short & fat also g- evidence of some g+c about in small cons. - colonies were not separated.

③ Uniform c, some short chains

MICROBIAL IDENTIFICATION

CODE NUMBER: MK
SUBJECT NAME: Martin Koesterer
SITE: 2 - Throat
SWAB: MOIST - _____ DRY - ✓
MEDIUM: N/A
DATE: 8/9/71 TIME: 1340

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3-4 - Poor dispersion of colonies difficult to find isolated colonies -

AROMA (IF PRESENT)

~~DESCRIPTION:~~

Neomycin 30 - Apparent inhibition of white, opaque colonies in 3 exceptions (exceptions not sampled for testing)

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1 a	① g+c in pairs, chains, clumps, singles, uniform size	Pos ②	ND	white	2	1	3	1	2
1 b	② g+ diplococci small	Neg	ND	colorless	2	1	3	2	1-3
1 c	③ g+rod pleomorphic	Neg	ND	Ivory	4	4	5	1	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							Staph / micrococcus
1-b							Staph / hyphococcus
1-c							2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

① very sparce slide, not overcrowded w organisms - those there exhibit practically every type of arrangement possible

② Shows tendency to decolorize easily, mostly singles (in diploid configuration), some short chains.

③ few organisms on slide - some appear as gram variable - spaces between stained areas on wd, gram variable as to size

MICROBIAL IDENTIFICATION

CODE NUMBER: MK
SUBJECT NAME: Martin Koestener
SITE: 2 - Nares
SWAB: MOIST - ✓ DRY - _____
MEDIUM - MB
DATE: ~~7/4/84~~ 7/5/84 TIME: 1340

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3 (1 type not accessable for testing at 24 hrs.)

AROMA (IF PRESENT)

~~DESCRIPTION:~~

Neomycin 30: complete inhibition surrounding disc

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2a	g+c in mostly clumps	Pos	ND	yellow-white	4	1	3	1	4
2b (2)	g+c mostly clumps	Pos	ND	white	2	1	3	1	4 (confirmed 48 hrs)
2c	g+ rod (as 2 c above)	Pos (3)	ND	grey	1	1	3	1	2
2d (4)	g var rod	~~Pos~~	ND	white	2	1	3	1	1-3

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							Staph
2-b							Staph
2-c							2.
2-d							corynebacterium

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

(2) Somewhat variable in size - much evidence of tendency to decolorize.

(4) Either overdecolorized or strongly gram variable Rods show much evidence of septum-like chains - need to look closely to observe all wall of rod.

MICROBIAL IDENTIFICATION

CODE NUMBER: MK
SUBJECT NAME: Martin Koestever
SITE: 3 - Skin (forearm)
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/9/71 TIME: 1310

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT ✓

Too few colonies to determine reaction positively

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) NA

DESCRIPTION:
Novobiocin 30 - Too few! - (no colony growth)

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3a	① Very large cocci	Pos	NT	white	2	1	3	1	±*
3b	much debris-no cont. / bact. organisms			colorless	4	4	3	2-3	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3a							Staph
3b							None

* 48 hrs - some colonies show beta hemolytic halo around; others of same apparent shape + color do not.

① Very large spheres, mostly clumps, but some chains. Some in 1's, 2, and 4's, uniform size

MICROBIAL IDENTIFICATION

CODE NUMBER: J.M.
SUBJECT NAME: John Mangialardi
SITE: 1 - Throat
SWAB: MOIST - _____ DRY - ✓
MEDIUM - N/A
DATE: 8/9/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) N/A

~~DESCRIPTION:~~ Neomycin 3S - inhibition of white colonies, no apparent effect on colorless ones.

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1a	(1) G+c clumps, 1S+ 2S	Neg (2)	ND	white	2	1	3	1	2
1b	(2) G+ diplococci	Neg	ND	colorless	2	1	3	2	1-3

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							Strep/Aerococcus
1-b							Strep/diplococcus

(1) Evidence of variable size & shape; also tendency to show some evidence of decolorization

(2) some short chains, organisms generally small and oval shape

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

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MICROBIAL IDENTIFICATION

CODE NUMBER: 1M

SUBJECT NAME: John Mangiacardi

SITE: 2- Nares

SWAB: MOIST - V DRY - _____

MEDIUM - NB

DATE: 8/9/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT V

TAXO P - PRESENT _____ ABSENT V

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) N/A

~~DESCRIPTION~~:

Neomycin 30 — complete inhibition around N 30 disc

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2a	(1) g+c mostly clumps	Pos	ND	white	2	1	3	1	2
2b	(2) g+ rod pleomorphic	N/eg	ND	white	1	1	2	2	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2a							Staph coagulase +
2b							

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(1) Strong tendency to decolorize, much variability in size, few singles

(2) Strongly g+, very variable in shape: small ovoid rods to large (3-4 x 1 μ) club-shaped rods.

(2a) Additional staining of Saprimin only made one strain colony after 48 hr. incub. showed large, clear areas within the rod (taken not to be spores by virtue of the size in relation to rod in which it existed) many club-shaped rods visible.

MICROBIAL IDENTIFICATION

CODE NUMBER: J. M.
SUBJECT NAME: John Mangialandi
SITE: 3 - Skin (forearm
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/9/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION: Too few to tell may be apparent in Sub. Lin

TAXO A - PRESENT _____ ABSENT _____

TAXO P - PRESENT _____ ABSENT _____

NUMBER OF APPARENT COLONY TYPES: _____

AROMA (IF PRESENT) N/A

~~DESCRIPTION:~~ Neomycin 30 - Too few to tell

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3a	No endospore Gram + Rods	Neg	ND	Colorless	4	3	3	2	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3a							None

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: J.S.
SUBJECT NAME: John Schelkopf
SITE: 1 - Throat
SWAB: MOIST - _____ DRY - ✓
MEDIUM - N/A
DATE: 8/9/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:
TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT) N/A

DESCRIPTION:
Neomycin 30 - inhibition of white & ivory opaque types - not of colorless ones

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1a	① G+ Cocci mostly singles, 2's, 4's some chains	Neg	ND	Colonies large white	1-2	4	3	2	1-3
1b	② G+ c clusters & singles 2's, uniform size	Neg	ND	white	2	1	3	1	2
1c	③ G+ c very large, mostly 1's & 2's some clusters	Pos	ND	Ivory	2	1	4	1	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							Strep / diplococcus
1-b							Strep / pneumococcus
1-c							Staph / micrococcus

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① cocci in the chains are smaller than those appearing in the singles or 2's, singles which appear large may be diplococci
② large cocci in all arrangements
③ also some long chains (10-15)

MICROBIAL IDENTIFICATION

CODE NUMBER: J. S.
SUBJECT NAME: John Shellcoff
SITE: 2 - Nares
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/9/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24) HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓ ? Perhaps inhibition
TAXO P - PRESENT _____ ABSENT ✓ ? ? colonies in halo (hemolysis) surrounding

NUMBER OF APPARENT COLONY TYPES: 3 difficult to say in 24 hrs.

AROMA (IF PRESENT) N/A

DESCRIPTION:

Neomycin 30 — complete inhibition of all growth except 1 β-hem. colony (micro)

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Entire plate looks like inoculum in oblique light & visual inspection only. However, transmitted light shows some clearing in halos (hemolysis?) + others w/o, some small pinpoint

DESCRIPTIVE KEYS: whitish colonies

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2a	g+c mostly clusters prim. uniform size	Pos		white	2	Halo	3	1	1-4 ?
2b	g+c as above	Pos		white	2	1	3	1	2
2c	g+c as above	Neg		colorless	1-4	1	3	2	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							Staph
2-b							Staph/anaerobes
2-c							Staph/aerobes

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: J S.
SUBJECT NAME: John Scheltopf
SITE: 3 - Skin (forearm)
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/9/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-(24) HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT ✓ ABSENT _____

TAXO P - PRESENT ✓ ABSENT _____

Apparently - although h
Very few colonies

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) N/A

~~DESCRIPTION:~~

Neomycin 30: No growth around disc

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

only one large white colony visible and
different. very colorless colonies at periphery
of main inoc. area.

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3a	+c mostly 1's + 2's some tetrad	Pos		white	1	1	3	1	2
3b	M. cinderea / Bact.	Neg		colorless	4	3	3	2	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3-a							Staph / micrococcus
3-b							none

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: E G

SUBJECT NAME: Ed Glanffield

SITE: L- Throat

SWAB: MOIST - _____ DRY - ✓

MEDIUM - _____

DATE: 8/3/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2-3 ?

AROMA (IF PRESENT)

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

white opaque colonies
colorless α & α/β hemol. - same organism ?

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a	G+ Cocci singles and Chains	Neg	ND	white	2	1	4	1	2
1-b	G+ Diplococci singles Neg Sing	Neg	ND	colorless	4	1	3	2	1-3
1-c ?	G+ Cocci mostly pairs some Chains	Neg	ND	"	4	1	3	2	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							Strep
1-b							strep/diplococcus
1-c							Strep/aureococcus

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: E G
SUBJECT NAME: Ed Glanfield
SITE: 2 - Nose
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/3/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 1

AROMA (IF PRESENT) NA

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
large white colonies.

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a	G+ cocci large Clumps	Pos	ND	white	2	1	4	1	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							Staph/micrococcus

MICROBIAL IDENTIFICATION

CODE NUMBER: E G
SUBJECT NAME: Ed Manfield
SITE: 3 - forearm, Skin
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/3/71 TIME: 1345

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) NA

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
Large white colonies
tiny colorless colonies

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3-a	G+ cocci large clumps			white	2	1	3	1	2
3-b	G+ cocci single & some chains			colorless	1	1	3	2	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3-a							Staph
3-b							Staph

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: JG

SUBJECT NAME: John Cooling

SITE: 1-Throat

SWAB: MOIST - _____ DRY - ✓

MEDIUM - —

DATE: 8/3/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT)

DESCRIPTION: NA

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Dry appearance - Some large colonies w yellow centers - rest white - small colonies = α hemol.

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a	G+ cocci mostly single some chains	Neg	ND	white yellow	2	1	4	1	2
1-b	G+ diplococci some in chains	Neg	ND	color less	2	2	3	2	1-3

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positi
2. Negati
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							Strep/gamococcus
1-b							Strep/diplococcus

MICROBIAL IDENTIFICATION

CODE NUMBER: SG
SUBJECT NAME: John Geating
SITE: 2-Nose
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/3/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:
TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT ✓
NUMBER OF APPARENT COLONY TYPES: 3
AROMA (IF PRESENT) NA
DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
Large white colonies some irreg, some tiny colorless colonies white to yellow centers

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a	+ cocci chains single	Pos	ND	white	2	1	4	1	2
2-b	" " chains*	Neg	ND	ivory	2	1	4	1	2
2-c	" " chains	Neg	ND	colorless	1	1	3	2	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							Staph
2-b							Strep
2-c							Strep

* many over-destained (decolorized)

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: SG
SUBJECT NAME: John Geoffing
SITE: 3 - Forearm, Skin
SWAB: MOIST - ✓ DRY - _____
MEDIUM - NB
DATE: 8/3/71 TIME: 1330

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:
TAXO A - PRESENT _____ ABSENT ✓
TAXO P - PRESENT _____ ABSENT ✓
NUMBER OF APPARENT COLONY TYPES: 2
AROMA (IF PRESENT) NA
DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
opaque colonies w yellow centers
" " Ivory color

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3-a	G+ cocci Clumps	Neg	ND	white to yellow	2	1	4	1	2
3-b	" " Short Chains	Neg	ND	Ivory	2	1	4	1	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3-a							Strep/Aerococcus
3-b							Strep/Aerococcus

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positiv
2. Negativ
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: SH

SUBJECT NAME: Stacy Hunt

SITE: 1-Throat

SWAB: MOIST - _____ DRY - ✓

MEDIUM - —

DATE: 8/3/71 TIME: 1315

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 3

AROMA (IF PRESENT)

DESCRIPTION: Non-specific

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTO-OXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
1-a	G+ cocci clumps	Neg	ND	white opaque	2	4	4	1	2
1-b	G+ diplococci some short chains	Neg	ND	clear	4	1	4	2	1-3
1-c	G+ cocci mostly singles short chains	ND	ND	~~Dark~~ clear	2	1	4	2	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
1-a							Strep
1-b							Strep/Diplococcus
1-c							Strep

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

ND Not Done

MICROBIAL IDENTIFICATION

CODE NUMBER: SH

SUBJECT NAME: Stacy Hunt

SITE: 2 - Nose

SWAB: MOIST - ✓ DRY - _____

MEDIUM - NB

DATE: 5/3/71 TIME: 1315

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION:

TAXO A - PRESENT _____ ABSENT ✓

TAXO P - PRESENT _____ ABSENT ✓

NUMBER OF APPARENT COLONY TYPES: 2

AROMA (IF PRESENT) NA

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)
Large white colonies — smooth, shiny, irreg. edge + 2 large crusted colony

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
2-a	G+ cocci in clumps & some short chains	Neg	N-D	white & yellow	2	1	4	1	2
2b	G+ mycelium	Neg	N-D	white	2	6	3	1	2

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg.	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
2-a							Staph
2-b							Candida

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

MICROBIAL IDENTIFICATION

CODE NUMBER: SH

SUBJECT NAME: Stacy Hunt

SITE: 3 - forearm, skin

SWAB: MOIST - ✓ DRY - _____

MEDIUM - NB

DATE: 8/3/71 TIME: 1315

PRIMARY CULTURE INFORMATION

RESULTS ON BLOOD AGAR (18-24 HOURS)

TAXO DISC REACTION: growth too sparse to fully evaluate reaction

TAXO A - PRESENT ____ ABSENT ____

TAXO P - PRESENT ____ ABSENT ____

NUMBER OF APPARENT COLONY TYPES: 1

AROMA (IF PRESENT) NA

DESCRIPTION:

OVERALL DESCRIPTION OF CULTURE: (COLOR, ETC.)

Very sparse growth - small translucent colonies visible, few only

SECONDARY CULTURE INFORMATION

SUBCULTURE NUMBER	A GRAM STAIN REACTION & CELLULAR MORPHOLOGY	BIOCHEM. CATALASE	BIOCHEM. CYTOOXIDASE	COLONY CHARACTERISTICS B FORM	C COLOR	D EDGE	E ELEVATION	F OPTICAL CHAR.	G HEMOL.
3-a	*G+ cocci - single or short chains			cylar-less	2	1	3	2	2

DESCRIPTIVE KEYS:

A. CELLULAR MORPHOLOGY
1. Cocci
2. Diplococci
3. Rods
4. Commas
5. Spirals
6. Branched Rods
7. Mycelium
8. Chlamdospores

B. COLOR
As Noted

C. FORM
1. Punctiform
2. Circular
3. Filamentous
4. Irregular
5. Rhizoid
6. Spindle

D. EDGE
1. Entire
2. Undulate
3. Lobate
4. Erose
5. Filamentous
6. Curled

E. ELEVATION
1. Effuse
2. Flat
3. Raised
4. Convex
5. Umbonate

F. OPTICAL CHARACTERISTICS
1. Opaque
2. Translucent
3. Opolescent
4. Irridescent

G. HEMOLYSIS
1. Positive
2. Negative
3. Alpha
4. Beta

SUBCULTURE NUMBER	ANTIBIOTIC SENSITIVITY/ZONE DIAMETER AM 10 mcg	P 10 u	N 30 mcg	G 2 mg	F/M 300 mcg	Te 30 mcg	PRESUMPTIVE I/D
3-a							Staph

* Appearance indicated possibility of over decolorization - organisms were large ovoid bodies in short chains - possibility of G- short rod.

6.5 COMMERCIAL PRODUCTS APPLICABLE TO THE I/D KEY